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Increased chemical stability but decreased physical protection of soil organic carbon in response to nutrient amendment in a Tibetan alpine meadow

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ABSTRACT

Nutrient amendment increases plant productivity but the effects and mechanisms on soil organic carbon (SOC) accumulation and stability remain unclear, especially in nutrient deficient alpine ecosystem. Here, based on an experiment combining nitrogen (N) and phosphorus (P) input continuously for 15 years, we found that nutrient amendment did not affect total SOC content, but increased mineral-associated C with decreasing soil aggregate stability. Despite increased total phospholipid fatty acid (PLFA) and bacteria PLFA, nutrient amendment decreased soil enzyme activities involved in C cycling. The ^{13}C NMR analyses showed that nutrient amendment decreased the aliphaticity but enhanced aromaticity of SOC. Structural equation models indicated that P availability (Olsen-P content) was most related to shifts in microbial community composition and decreased enzyme activities. Moreover, the concomitantly reduced aggregation and increased mineral-associated C were mainly attributable to the decrease of fungal biomass and increase of bacterial biomass. Together, interconnected factors such as increased acidity, aggregate destabilization, microbial community shift towards bacteria, and loss of oxidative enzyme activities could contribute to the overall response of SOC under intensive N and P input. In particular, available P rather than N may re-shape the pattern of physical and chemical stabilization of SOC, shifting from moderately physical protection to highly chemical stability, implicating the pivotal roles of P management in C cycling of alpine ecosystem.

1. Introduction

Permafrost soils store vast quantities of soil organic carbon (SOC; 1, 320 ± 200 Pg) and are of increasing concern because of their potential positive feedbacks to climate warming (Ding et al., 2017). The Tibetan Plateau has the largest extent of alpine permafrost ecosystem in the world. The rate of temperature rises in this region (0.3–0.4 °C per decade) is about twice as high as the global warming rate (IPCC, 2013), which may accelerate soil C decomposition, and further intensifies global warming. Moreover, increased mineralization of soil organic matter can enhance nutrient release, especially inorganic nitrogen (N) and phosphorus (P) (Mack et al., 2004). Studies also have observed atmospheric N deposition in the Qinghai–Tibetan Plateau, ranging from 0.11 to 0.20 kg N ha^{-1} (Liu et al., 2013). In addition, increased nutrient input could considerably alter critical ecosystem processes in this region, such as plant production and decomposition, which in turn affect SOC dynamics. Therefore, a source or sink for atmospheric CO_2 in such alpine ecosystem depends on the balance between plant production and SOC decomposition, and the relationship between nutrient availability and C storage remains uncertain.

Concerns over N input or deposition on SOC dynamics have highlighted that N enrichment generally leads to an increase in aboveground plant biomass and accumulation of organic matter detritus in top soils (LeBauer and Treseder, 2008), but exerts positive (Fornara

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et al., 2013; Zak et al., 2017), negative (Li et al., 2016; Khan et al., 2007) or negligible (Silveira et al., 2013; Brown et al., 2014) effects on total SOC content. The contrasting effects of N inputs on different soil C pools could be attributed to interrelated responses of plants, microbes and soil properties and a model simulation recently confirmed that the impact of N inputs on soil C dynamics was controlled by microbial physiology, soil mineralogy and acidity (Averill and Waring, 2018).

As soils warm in response to climate change, not only N but also P mineralization from soil organic matter is expected to increase (Mack et al., 2004). Studies that seek to predict how N input impact SOC dynamics need to consider how changes in the availability of soil P will modify N input effects. The addition of P alone also has the potential to directly alter SOC storage. For example, Bradford et al. (2008a,b) found that soils amended with P could increase plant biomass and then enhance the sequestration of fresh plant-C inputs to soils, while P input could also increase decomposition of stable SOC. Changes in soil P availability can also modify ecosystem responses to N deposition by modulating microbial community, particularly the arbuscular mycorrhizal fungi (Camenzind et al., 2018; Treseder et al., 2018), thus in turn affected plant C allocation belowground and macroaggregation process (Liu et al., 2012). However, mechanisms of the N and P enrichment on SOC dynamics are still unclear.

Although the increase in nutrient availability could typically increase total SOC content due to an increase in the production of plant residues in nutrient-limited ecosystems, the net effect on the soil C storage depends on the balance between newly transformed C from plant residues and decomposition losses from the existing C (Averill and Waring, 2018; Ye et al., 2018). First, nutrient amendment may alter litter chemistry, which is a primary controller of litter decomposition and SOC chemical composition (Allison et al., 2013). Second, nutrient amendment may change microbial community composition and activity, and hence affect litter transformation and SOC formation (Wang et al., 2014; Jian et al., 2016). Third, nutrient amendment may also affect soil aggregate stability and soil mineral availability via changing plant root distribution and soil pH (Miller and Jastrow, 1990; Yu et al., 2017). The relative strengths of these biotic and abiotic responses would determine the net effect of nutrient amendment on aggregate-occluded C and organo-mineral associations. Emerging evidence showed that physical protection by aggregation and the adsorption of C to minerals are the key mechanisms of SOC persistence (Schmidt et al., 2011). To date, there is a lack of systematic research combining physicochemical and biological mechanisms of SOC accumulation and stabilization.

The ecological consequences of nutrient enrichment on the degradation of ecosystem structure and functioning are still being concerned (Suding et al., 2005; Ding et al., 2017). Although the discrepancies exist, overdose of chemical nutrients generally reduces soil biodiversity and simplifies soil food web, and the changes of microbial community composition can have far-reaching functional feedbacks to climate change (de Vries et al., 2013). Considering SOC-associated functioning is a foundation of multiple ecosystem services, the mechanistic understanding SOC stabilization in response to nutrient management will be critical to ecologically intensive management (Paustian et al., 2016). Taking advantage of a long-term N and P enrichment experiment, our objectives were to (i) investigate the effects of long-term N and P inputs on various C pools and (ii) develop a mechanistic understanding of SOC stabilization in a comprehensive way via integrating soil microbial, physical and biochemical properties. We hypothesized that long-term nutrient amendment would (i) alter soil microbial community composition and activity, i.e. increase bacterial biomass, reduce fungal biomass and related oxidase activity, due to the aggravation of acidification and nutrient enrichment; (ii) increase decomposition of soil organic C by increasing the accessibility of organic carbon protected in soil macroaggregates to microbial utilization, leading to a shift of SOC from aggregate protection to chemical recalcitrance or mineral-associated C (Fig. 1).

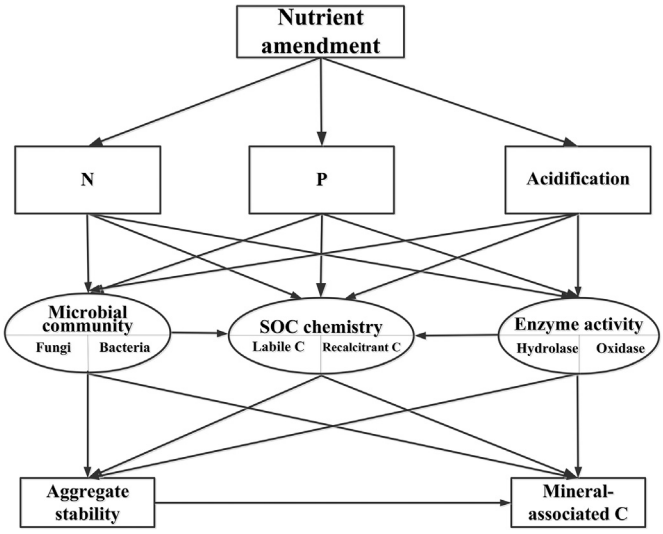

Fig. 1. Proposed conceptual diagram of nutrient amendment affect SOC chemical composition, aggregate and mineral-associated C.

2. Materials and methods

2.1. Site description

A long-term experiment was established at the research station of alpine meadow and wetland ecosystems of Lanzhou University (35°58′N, 101°53′E; with an altitude of 3500 m a.s.l.), on the eastern Tibetan Plateau. The humid-alpine climate is characterized by a mean annual precipitation of 620 mm and a mean annual temperature of 1.2 °C. The soil at the site is clay loamy-sand texture (clay 18%, silt 14% and sand 68%). The topsoil layer (0–20 cm) of the unmanaged meadow contained 36.54 g kg^{-1} organic C, 3.56 g kg^{-1} total N, 4.94 mg kg^{-1} available (Olsen) P, 127.41 mg kg^{-1} exchangeable K, and had a pH (H_2O) of 7.64. The native vegetation types are Arctic alpine and Chinese Himalayan plants, including clonal *Kobresia graminifolia Clarke.*, *Poa botryoides Trin.*, *Elymus nutans Griseb.* and *Anemone rivularis Buch.-Ham. ex DC*. The abundance of plant species reaches 20–35 per 0.25 m^2, and mean above-ground biomass is 280–400 g m^{-2} (dry mass) (Luo et al., 2006; Yang et al., 2011; Liu et al., 2012; Guo et al., 2017).

2.2. Experimental design and sampling

The experiment was established at a flat area and was fenced-off. The field was divided into 25 plots with little inter-plot variation, and each plot was 60 m^2 (6 × 10 m). The plots were arranged in a regular five by five matrix and isolated from the buffer zones (1 m wide) without fertilization. Five nutrient amendment levels with five replicates were established in 1999. Ammonium phosphate $(NH_4)_2HPO_4$ was applied in mid-May annually during the growing season at the rate of 0 (NP0), 30 (NP30), 60 (NP60), 90 (NP90) or 120 (NP120) g m^{-2} yr^{-1}, equivalent to 0, 6.3, 12.7, 18.9, 25.2 g N m^{-2} yr^{-1} and 0, 7.0, 14.1, 21.1, 28.2 g P m^{-2} yr^{-1}.

In this study, we chose four treatments from this fertilization experiment, i.e., NP0, NP30, NP90 and NP120. Soil samples were collected in mid-July 2015, five soil cores (5 cm diameter, 20 cm depth) were taken randomly from each plot then combined to form one composite sample. These soils were immediately transported to the laboratory and then were passed through a 1 mm sieve to remove large roots and rocks. Then a subsample of the soil was kept at 4 °C used for microbial analyses and the other subsample was air dried for physicochemical analysis.

2.3. Determination of soil properties

Soil pH was measured in a 1:2.5 ratio of soil to deionized water with pH meter. The content of SOC was determined by the Walkley and Black dichromate oxidation method (Nelson and Sommers, 1996). Total N content was determined following micro-Kjeldahl digestion (Bremner et al., 1996). Soil inorganic nitrogen (NH_4^+—N and NO_3^--N) were extracted with 50 ml of 2 M KCl followed by determined using a continuous flow analyzer (Skalar, Breda, Holland). Soil total P was measured using H_2SO_4 fusion with phosphate detection in neutralized extracts at 880 nm by automated molybdate colorimetry using a Shimadzu ultraviolet spectrophotometer. Available phosphorus (AP) content was determined colorimetrically using molybdate after extracting samples with 0.5 M $NaHCO_3$ (Olsen et al., 1954). The dissolved organic carbon (DOC) was exacted from 10 g fresh soil using 50 ml ultrapure water by centrifugation (8000 rpm, 10 min). The filtrate that passed through a 0.45 μm filter membrane was analyzed with a total C analyzer (Elementar, Germany).

The microbial community composition in soil samples was measured by PLFA analysis according to Bligh and Dyer (1959) with minor modification. In brief, fatty acids were extracted from 8.0 g freeze-dried soils with chloroform-methanol-citrated buffer mixture (25 mL at a 1:2:0.8 vol bases). The phospholipids in the organic phase then were separated from neutral lipids and glycolipids using solid phase extraction (SPE) tubes (ANPEL Laboratory Technologies Inc., China). The fatty acid methyl esters were analyzed in an Agilent 6850 series Gas Chromatograph after mild alkaline methanolysis. The PLFAs chosen to indicate bacterial biomass were i14:0, i15:0, a15:0, i16:0, 16:1ω7c, i17:0, a17:0, 17:0cy, 18:1ω9, 18:1ω7c and 19:0cy, while PLFA 18:2ω6.9c was used to indicate fungal biomass and PLFA 16:1ω5c was used as specific indicator for arbuscular mycorrhizal fungi (AMF) (Frostegård and Bååth, 1996; Bossio and Scow, 1998; Mikola and Setälä, 1998). For each sample, the abundance of PLFAs was expressed as fatty acid nmol g^{-1} dry soil.

2.4. Aggregate and SOC fractionation

To achieve the functionally distinct SOC pools, soils were separated using the wet sieving and size density fractionation approach modified from Six et al. (2010) and Plaza et al. (2013). Briefly, 50.0 g air dried soil was spread on a 250 μm sieve and dispersed with 200 ml deionized water for 5 min in 1 L beaker at room temperature, subsequently moving the sieve up and down 3 cm with 100 repetitions during a period of 5 min. The fraction remaining on the sieve was washed into a petri dish, and weighed after drying at 60 °C for 24 h. Water plus remaining soil in beaker was poured onto a 53 μm sieve and repeating the process. Three aggregates size classes were obtained: macroaggregate (> 250 μm), microaggregate (250-53 μm) and the silt and clay fraction (< 53 μm).

To obtain all free light fraction (Free-LF) and to extract all the silt and clay fraction, a 5.0 g subsample of macro- or micro-aggregates was further separated by density flotation in 1.8 g cm^{-3} sodium iodide. The floating fraction (Free-LF) was washed with deionized water for 5 times. The heavy fraction was centrifuged for 20 min at 1, 250 g (2, 500 rpm) after adding 50 ml deionized water, and then supernatant liquid was immediately poured out. The above procedures were repeated 5–6 times to eliminate the interference of sodium iodide. The material heavier than 1.8 g cm^{-3} was transferred to a 53 μm sieve and shaken with 20 glass beads (4 mm diameter). A continuous and steady water flowing through the sieve made the organic matter protected in aggregates disperse, releasing occluded particulate organic matter (O-POM) and the silt and clay fraction. All the fractions were dried at 60 °C for 24 h and weighed. Finally, we obtained four physical fractions: Free-LF, macroaggregate O-POM, microaggregate O-POM and silt and clay fraction. We interpreted C in the silt and clay fraction as predominantly representing mineral-associated C because density fractionation has removed all light fraction (Stewart et al., 2008).

2.5. Solid-state ^{13}C NMR spectroscopy

Solid-state ^{13}C NMR spectroscopy was performed in organic carbon of soil. In order to improve the signal-to-noise ratio, the hydrofluoric acid (HF) treatment was conducted to remove paramagnetic compounds and to increase the organic C contents. 5.0 g air-dried soil samples placed in 100 ml centrifuge tube and was saturated with 50 ml of 10% HF solution. The samples were centrifuged for 10 min at 1, 800 g (3, 000 rpm) after vibratory agitation, the supernatant was poured out and replaced with fresh HF solution. This successive procedure was repeated 8 times, and the periods of shaking were 1 h (four times), 12 h (three times) and 24 h (once), respectively. Each sample was rinsed 5–6 times with deionized water to remove the residual HF solution after the last treatment. Subsequently, the soil samples were freeze-dried in the CHAIST (Alpha 1–4 LDplus, GERMANY) to be analyzed. The cross-polarization magic angle spinning (CPMAS) technique was obtained on a bruker Avance III WB 400 MHz in a magnetic field strength of 9.4 T corresponding to the Larimore frequency of 100.6 (^{13}C), 4 mm rotors (scan number = 5, 000, spin rate = 10 KHz).

The spectrums of each sample were divided into four main functional areas including alkyl C (0–45 ppm), O-alkyl C (45–110 ppm), aromatic C (110–160 ppm) and carbonyl C (160–220 ppm). The relative intensities of different C functional groups were obtained by integrating the area below the curves. Solid-state ^{13}C NMR spectra statistical analyses were done with MestReNova software.

To facilitate interpretation of the ^{13}C NMR spectra, two indicators of the stability of soil organic carbon were calculated: the ratio of alkyl C/O-alkyl C (Aliphaticity) which is used as an indicator of organic carbon decomposition potential, aromatic-C/(alkyl C + O-alkyl C + aromatic C) ratio (Aromaticity) which is used as an indicator of the advanced stages of decomposition.

2.6. Enzyme activity

The activities of C-hydrolase (α-glucosidase, β-glucosidase, cellobiohydrolase, β-xylosidase), N-hydrolase (L-aminopeptidase) and P-hydrolase (phosphatase) were measured using microplate fluorometric assay according to the protocol of Bell et al. (2013). Soil homogenates were prepared by adding 2.75 g soil to 91 ml of 50 mM Tris buffer (pH 7.5) in a blender for 1 min. Soil suspension of 800 μl were then added to 96-well deep well plates along with 200 μl of 200 mM fluorometric substrates in each well with three replicates. After incubation in the dark for 4 h at 25 °C, the plates were centrifuged for 3 min at 2, 960 g (4, 800 rpm), and 250 μl of soil supernatant were transferred from each well into black flat-bottomed 96-well plates (into corresponding wells), then scanned on a TECAN infinite M200 Multifunctional microplate fluorometer (Grödig, Austria) with 365 nm excitation and 450 nm emission filters.

The other two oxidases phenol oxidases and peroxidases involved in recalcitrant C cycling were measured in a clear 96-well microplate using the substrate of L-3,4-dihydroxyphenylalanine and H_2O_2 (Allison and Jastrow, 2006). Briefly, 200 μl aliquots of homogenate were dispensed into replicate wells. For phenol oxidase, 50 μl of 20 mM DOPA is added to each well. Peroxidase assays receive 50 μl of 20 mM DOPA plus 15 μl of 0.3% H_2O_2. The microplates were incubated 25 °C for 18 h in the darkness and the activities were assayed using a microplate fluorometer (Tecan infinite 200, Switzerland) with 450 nm emission filters. The enzyme activities were expressed in mol g^{-1} dry soil h^{-1}.

2.7. Calculations and statistical analyses

Aggregate stability was expressed as mean weight diameter (MWD), calculated as the sum of the mass fraction remaining on each sieve, multiplied by the inter-sieve size accordance with the formula:

$$MWD = \sum d*m \quad (1)$$

where d is the mean diameter of the two sieves (mm); and m is relative fraction mass of aggregates (%).

Structural equation modelling (SEM) was conducted to analyze hypothetical pathways that may explain how nutrient amendment affects the dynamics of different SOC pools. First, analysis of variance (ANOVA) followed by Duncan's post hoc tests were used to test the effects of nutrient amendment on abiotic soil properties, and microbial parameters. Our results showed that nutrient amendment significantly affected mass proportions of aggregates and mineral-associated C but did not affect total SOC and O-POM. Thus, SEM was performed to explore the dominant mechanism resulting in changes of soil aggregate structure and mineral-associated C in response to nutrient amendment. Based on a priori and theoretical knowledge, we assumed that nutrient amendment alters soil abiotic properties, microbial community composition, enzyme activity and soil organic carbon chemical composition, which in turn affects soil aggregate stability and mineral-organic associations (Fig. 1). Before the SEM analysis, we reduced the number of variables for microbial community composition, enzyme activity and soil organic carbon chemical composition through Principal Component Analysis (PCA). The first principal components (PC1) were used in the subsequent SEM analysis to represent microbial community composition (PC1 explained 96.8% of the variation; Fig S1), enzyme activity (PC1 explained 94.1% of the variation; Fig S2) and soil organic carbon chemical composition (PC1 explained 90.6% of the variation; Fig S3). Data were fitted to the models using the maximum likelihood estimation method. Model fit was assessed by χ^2-text, the comparative fit index (CFI) and the root square mean error of approximation (RSMEA).

All univariate analyses were performed using SPSS 17.0 (SPSS, Chicago, IL, USA). PCA was performed using CANOCO Version 5.0 (Plant Research International, Wageningen, The Netherlands). SEM analysis was performed using "*lavaan*" package in R version 3.3.2 (Rosseel, 2012).

3. Results

3.1. Soil abiotic and biotic properties

Nutrient amendment significantly increased the contents of soil available P and total P but reduced soil pH and DOC content ($P < 0.05$; Table 1). The content of NO− 3-N was markedly higher at intermediate (i.e., NP90) and high levels of nutrient amendment (i.e., NP120) compared with low level of nutrient amendment (i.e., NP30) and control treatment (i.e., NP0). However, nutrient amendment had no significant impacts on NH+ 4-N, total organic C, total N and C/N ratio (Table 1). Regardless of nutrient amendment level, intermediate and high levels of nutrient amendments increased the contents of total PLFA, bacteria PLFA, Gram positive and Gram negative bacteria PLFA ($P < 0.05$; Fig. 2a, b, c and d), but reduced fungal PLFA, AMF and fungal to bacterial ratio ($P < 0.05$; Fig. 2e, f and g). Generally, the activities of C-hydrolases (i.e., α-glucosidase, β-glucosidase, cellobiohydrolase and β-xylosidase) and oxidase (i.e., phenol oxidases and peroxidases) were significantly decreased but N-hydrolase (i.e., L-aminopeptidase) and P-hydrolase (i.e., phosphatase) were increased with increasing rate of nutrient amendment ($P < 0.05$; Fig. 3). Intermediate and high levels of nutrient amendments decreased the activities of α-glucosidase and peroxidase (Fig. 3a, f). Activities of β-xylosidase and phenol oxidases decreased with increasing levels of nutrient amendment ($P < 0.05$; Fig. 3d and e). However, higher levels of nutrient amendments accompanied with higher phosphatase activity (Fig. 3h).

3.2. Soil aggregate distribution and organic C in different fractions

In comparison with control treatment, intermediate and high levels of nutrient amendments significantly decreased the mass proportion of macroaggregates, resulting in a decrease in MWD ($P < 0.05$; Fig. 5). The C content of free light fraction was significantly lower in low and high levels of nutrient amendment than in control treatment ($P < 0.05$; Fig. 4). Nutrient amendment increased the content of mineral-associated C but only high level of nutrient amendment had significantly higher mineral-associated C than control treatment ($P < 0.05$; Fig. 4d). However, there were no significant differences in macroaggregate O-POM and microaggregate O-POM among all treatments (Fig. 4b and c).

3.3. ^{13}C nuclear magnetic resonance spectroscopy

The integration of the major regions of ^{13}C NMR revealed that O-alkyl C and alkyl C were the dominant C components in soils (Fig. 6a and b). On average, nutrient amendment significantly decreased the relative intensity of alkyl C by 10% but increased aromatic C by 12% and carbonyl C by 4% (Fig. 6a, c and d), resulting in a 9.8% decrease in aliphaticity and 13.2% increase in aromaticity (Fig. 6e and f).

3.4. Controls on aggregate stability and mineral-associated C

The SEM model adequately fitted the data describing interaction pathways among soil abiotic and biotic properties, aggregate and mineral-associated C in response to nutrient amendment (Fig. 7). Nutrient amendment directly induced changes in soil available N and P and acidification, explaining 62%, 94% and 59% of the total variance, respectively. The pathway of P availability directly altered SOC composition ($R^2 = 79\%$), microbial community ($R^2 = 84\%$) and enzyme activity ($R^2 = 86\%$). Although microbial community had no direct effect on the SOC composition in SEM, it had a significant correlation with specific chemical components in linear regression analysis (Figs. S6 and S7). Soil acidification was positively related to the decreased enzyme activity. The reduced soil aggregate stability and increased mineral-associated C were mainly attributed to the changes in microbial community composition. Notably, soil P availability, rather than N

Table 1
Effects of different rates of N and P amendment on soil abiotic properties. Data are means ± standard errors (n = 5).

Parameter	NP0	NP30	NP90	NP120
Soil pH	7.21 ± 0.04^{a}	7.18 ± 0.15^{ab}	6.73 ± 0.31^{b}	6.50 ± 0.26^{c}
Total organic C (g kg^{-1})	56.0 ± 2.84^{a}	53.8 ± 2.56^{a}	53.3 ± 3.55^{a}	53.2 ± 1.80^{a}
Total N (g kg^{-1})	5.38 ± 0.29^{a}	5.23 ± 0.05^{a}	5.31 ± 0.31^{a}	5.08 ± 0.13^{a}
Total P (g kg^{-1})	0.89 ± 0.03^{d}	1.45 ± 0.04^{c}	2.29 ± 0.08^{b}	2.52 ± 0.10^{a}
C: N ratio	10.5 ± 0.79^{a}	10.3 ± 0.46^{a}	10.1 ± 0.43^{a}	10.5 ± 0.28^{a}
Dissolved organic carbon (mg kg^{-1})	281.9 ± 9.53^{a}	211.9 ± 9.10^{b}	224.2 ± 6.81^{b}	212.3 ± 10.7^{b}
NO− 3-N (mg kg^{-1})	3.29 ± 0.40^{b}	2.74 ± 0.25^{b}	7.25 ± 0.97^{a}	6.66 ± 0.72^{a}
NH+ 4-N (mg kg^{-1})	0.65 ± 0.05^{a}	0.62 ± 0.01^{a}	0.71 ± 0.09^{a}	0.64 ± 0.04^{a}
Available P (mg kg^{-1})	8.10 ± 0.66^{d}	28.3 ± 0.91^{c}	107.0 ± 5.36^{b}	154.5 ± 4.32^{a}

NP0, NP30, NP90 and NP120 represent the amendment rates of 0, 30, 90 and 120 g $(NH_4)_2HPO_4$ m^{-2} yr^{-1}, respectively. Different letters denote significant differences among different nutrient amendments ($P < 0.05$).

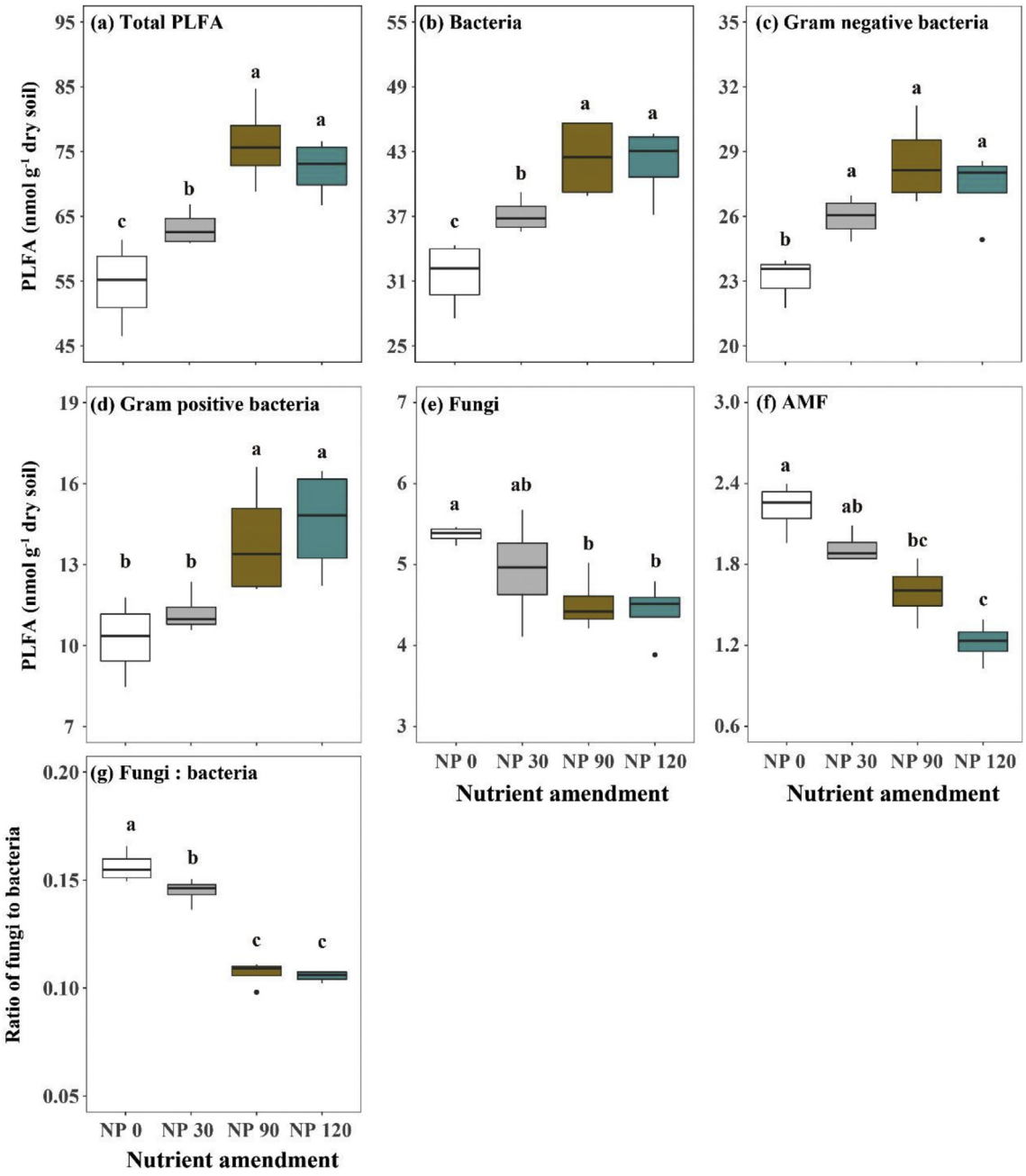

Fig. 2. Effects of different rates of N and P amendment on the contents of total phospholipid fatty acid (PLFA, a), bacteria (b), Gram negative bacteria (c), Gram positive bacteria (d), fungi (e), arbuscular mycorrhizal fungi (AMF, f) and fungi: bacteria ratio (g). NP0, NP30, NP90 and NP120 represent the amendment rates of 0, 30, 90 and 120 g $(NH_4)_2HPO_4$ m^{-2} yr^{-1}, respectively. Box plots represent the smallest observation, lower quartile, median, upper quartile, and largest observation (n = 5). Different lowercase letters indicate significant difference at $P < 0.05$ among different nutrient amendments.

availability, altered microbial community and then aggregate stability and mineral-associated C (Figs. 7 and 8 and S5).

4. Discussion

4.1. Nutrient amendment reduced aggregate stability

Long-term nutrient enrichment significantly reduced the proportion of macroaggregates and aggregate stability (Fig. 5), indicating that chemical fertilization weakened SOC physical protection. Fungal hyphae often play an important role in promoting aggregate formation (Rillig et al., 2002; Mummey, 2006). At our experimental site, an earlier study observed that extraradical hyphal length density of AMF decreased following N and P amendment (Liu et al., 2012; Xu et al., 2018). Our study also found a significantly negative relationship between P availability and fungi biomass (Figs. 2f, 7 and 8). Together, these results indicate that P-induced reduction in fungi biomass could depress soil macro-aggregate formation, which was further confirmed by our SEM results. Further, it was reported that increasing N inputs also reduced AMF abundance and shifted mycorrhizal functioning towards parasitism (Bradley et al., 2006; Jiang et al., 2018) which would reinforce negative effects of P on aggregation. In addition, plant roots could also promote aggregate formation (Six et al., 2004). Nutrient additions often reduce allocation of photosynthesis product to belowground and hence reduce plant biomass (Müller et al., 2000). Thus, the reduced aggregate stability may also attribute to reduced root biomass.

Modification in aggregation by SOC chemical composition have been previously reported (Piccolo and Mbagwu, 1999; Kavdır et al.,

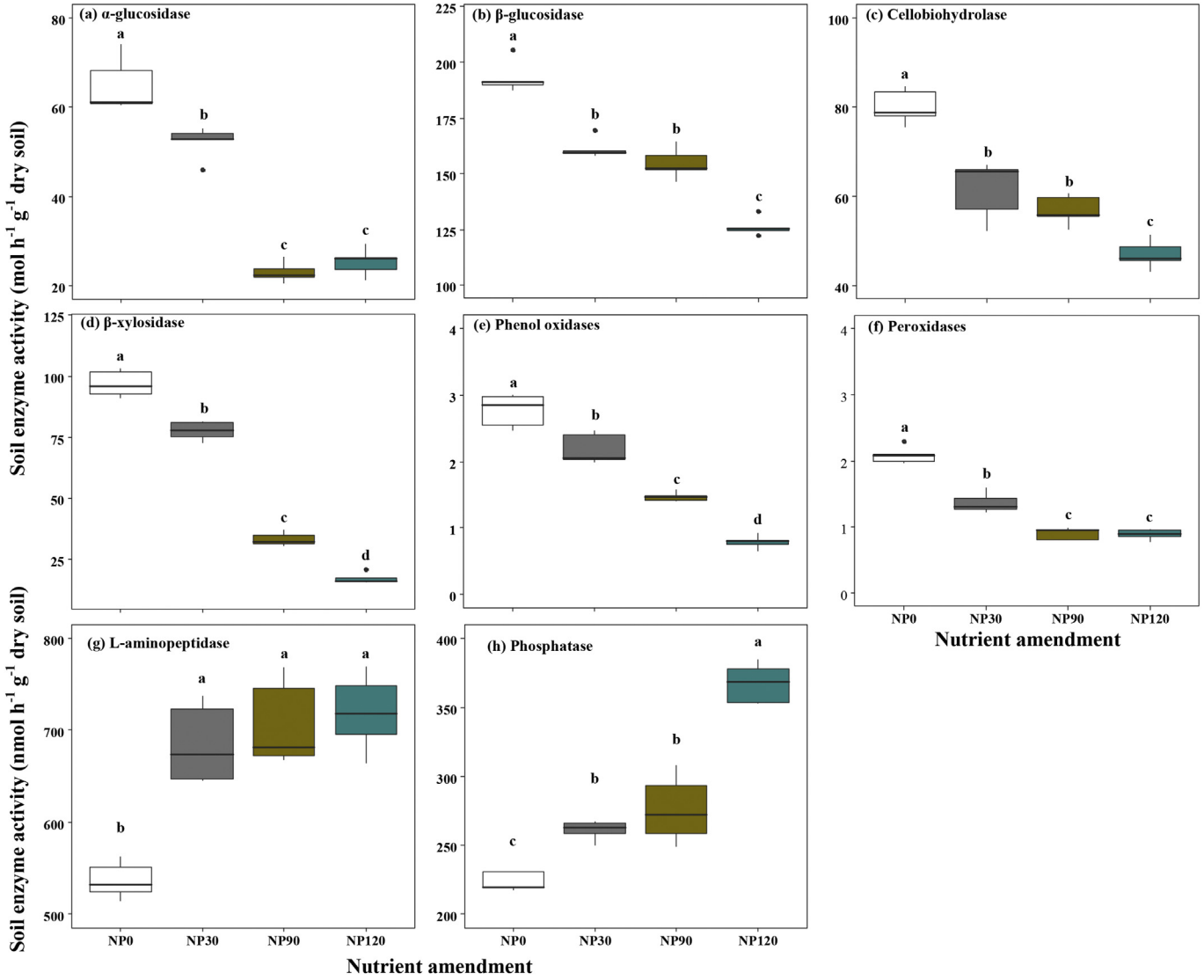

Fig. 3. Effects of different rates of N and P amendment on α-glucosidase (a), β-glucosidase (b), β-xylosidase (c), cellobiohydrolase (d), phenol oxidases (e), peroxidases (f), L-aminopeptidase (g) and phosphatase (h). Box plots represent the smallest observation, lower quartile, median, upper quartile, and largest observation (n = 5). Different lowercase letters indicate significant difference at $P < 0.05$ among different nutrient amendments. For treatment abbreviations see Fig. 2.

2005). While in this study, alkyl C was positively related to aggregate stability, aromatic C was negatively associated with aggregate stability (Fig. S8). At medium to long-term scale, the negative relationship between aromatic C fractions and aggregate stability possibly was due to the amphiphilic character of aromatic components, which might exhibit hydrophilic on exterior surfaces when forming aromatic-Fe complexes (Wershaw and Robert, 1999; Huang et al., 2018; Sarker et al., 2018). In addition, Spaccini et al. (2006) demonstrated that the degradation of soil structure might be related to the progressive loss of the cementing action of alkyl compounds in soil. Therefore, nutrient amendment weakened physical protection partly due to the alteration of chemical composition.

4.2. Nutrient amendment increased mineral-associated C

Although nutrient amendment did not affect total SOC, it decreased free light fraction C but increased mineral-associated C (Fig. 4), which indicated that separating total soil C into different C pools was useful for understanding soil C cycling processes (Neff et al., 2002). The light fraction consists of partially decomposed plant materials and by-products of decomposition (Boone, 1994) and the enhanced decomposition of light fraction C with added nutrient usually results from increasing photosynthesis inputs to soils (i.e. the priming effect). Similarly, Riggs et al. (2015) showed that N addition increased the decomposition of 'fast pool', but decreased that of the 'slow pool' of SOC in grassland. Our results also showed that the ratio of N to P significantly affected light fraction C (Table S1), indicating that the changes in P would potentially modify N effects.

We found that nutrient amendment increased mineral-associated C (Fig. 4), consistent with other nutrient addition studies (Neff et al., 2002; Bradford et al., 2008a; Cusack et al., 2011; Huang et al., 2018). Since nutrient amendment increased plant production and litter inputs over time in our study site (Luo et al., 2006; Liu et al., 2012) and inhibited oxidative enzyme activities (Fig. 2), the increased mineral-associated C could be attributed to the increased labile C transformation to the mineral-associated C and/or reduced decomposition losses of the existing mineral-associated C. Therefore, several mechanisms may contribute to the increased content of mineral-associated C. First, both linear regression and SEM results showed that increases in microbial biomass were statistically associated with strong increases in mineral-associated C (Fig. 7 and S6), consistent with the importance of microbial detritus as a key input to mineral-associated C pools (Cotrufo et al.,

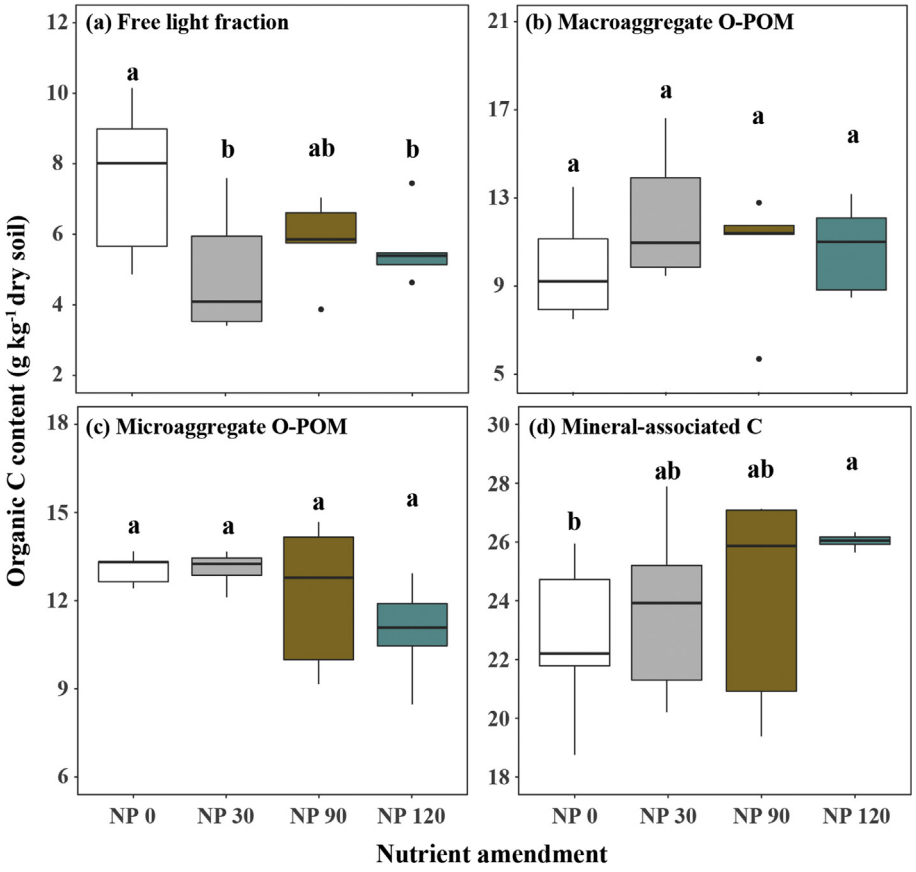

Fig. 4. Effects of different rates of N and P amendment on carbon distribution among free light fraction (a), macroaggregate O-POM (occluded-particulate organic matter, b), microaggregate O-POM (c) and mineral-associated C (d). Box plots represent the smallest observation, lower quartile, median, upper quartile, and largest observation (n = 5). Different lowercase letters indicate significant difference at $P < 0.05$ among different nutrient amendments. For treatment abbreviations see Fig. 2.

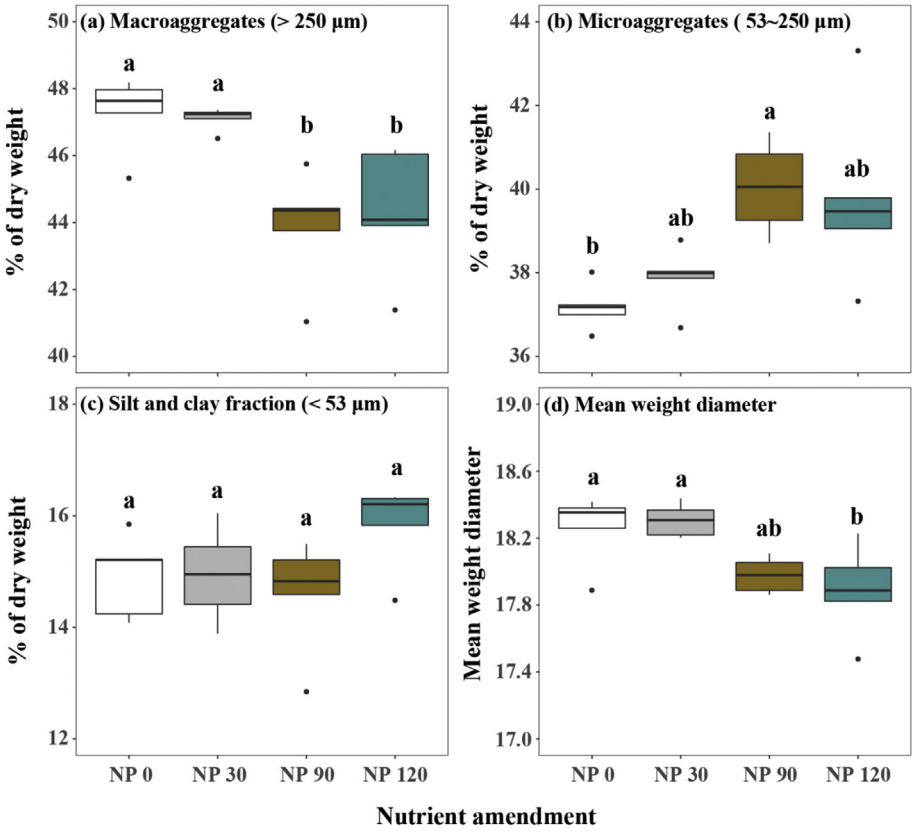

Fig. 5. Effects of different rates of N and P amendment on mass distribution of different aggregates and aggregate stability. Box plots represent the smallest observation, lower quartile, median, upper quartile, and largest observation (n = 5). Different lowercase letters indicate significant difference at $P < 0.05$ among different nutrient amendments. For treatment abbreviations see Fig. 2.

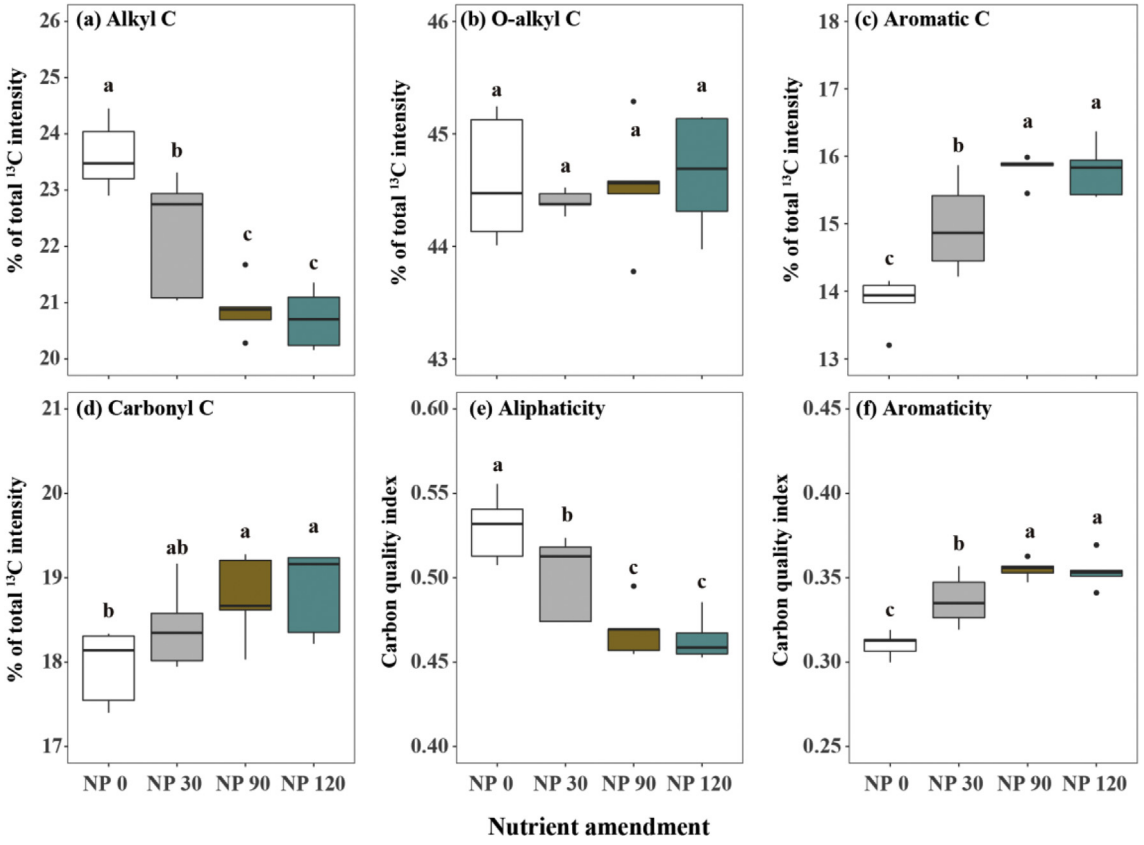

Fig. 6. Effects of different rates of N and P amendment on relative intensity distributions of the various bands in ^{13}C NMR spectra and carbon quality index. Box plots represent the smallest observation, lower quartile, median, upper quartile, and largest observation (n = 5). Different lowercase letters indicate significant difference at $P < 0.05$ among different nutrient amendments. For treatment abbreviations see Fig. 2.

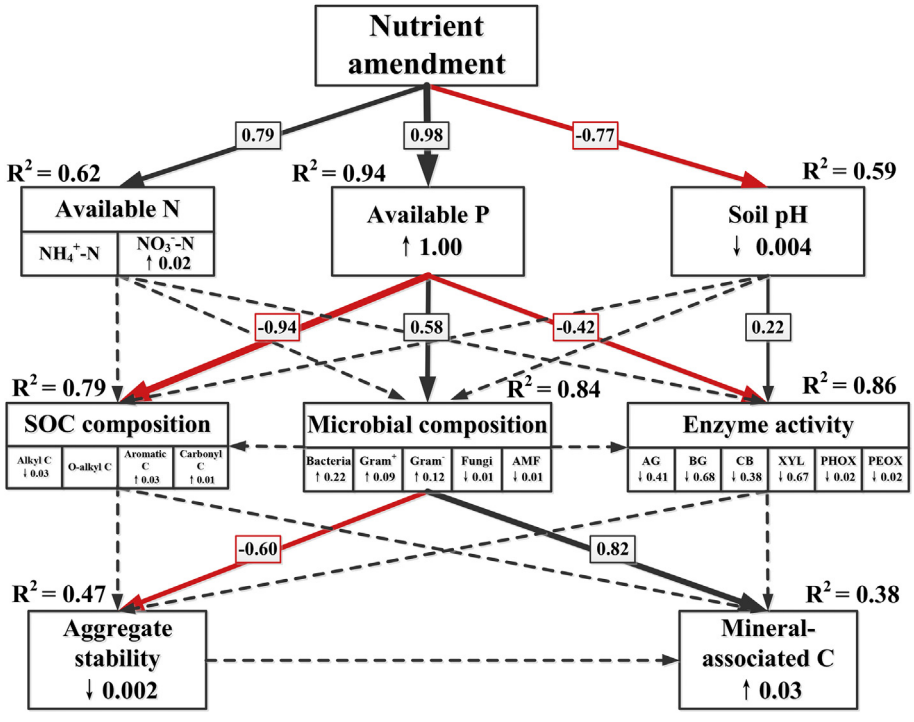

Fig. 7. Structural equation model (SEM) analysis of the contributions of different factors affected nutrient amendment on soil aggregation and mineral-associated C. Square boxes indicate variables included in the model: $Gram^+$, Gram positive bacteria; $Gram^-$, Gram negative bacteria; AG, α-glucosidase; BG, β-glucosidase; CB, cellobiohydrolase; XYL, β-xylosidase; PHOX, phenol oxidases; PEOX, peroxidases. The symbols "↑" and "↓" indicate a significant increase or decrease, respectively, in response to nutrient amendment. The number in each square box indicates the response to nutrient amendment (slope of linear model with nutrient levels as a continuous predictor, e.g., for available P, mg kg^{-1} per unit of $(NH_4)_2HPO_4$ m^{-2} yr^{-1}). Results of the optimal model fitting: Chi-square (χ^2) = 17.797, degree of freedom (*df*) = 15, $P = 0.273$, comparative fit index (CFI) = 0.989, root square mean error of approximation (RMSEA) = 0.097. Numbers at arrows are standardized path coefficients. Arrow thickness represents the strength of the relationships. Black arrows indicate significant positive relationships ($P < 0.05$) and red arrows indicate significant negative relationships ($P < 0.05$). Dashed arrows donate the non-significant effects ($P > 0.05$). Additionally, R^2 values associated with response variables indicate the proportion of variation explained by relationships with other variables. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

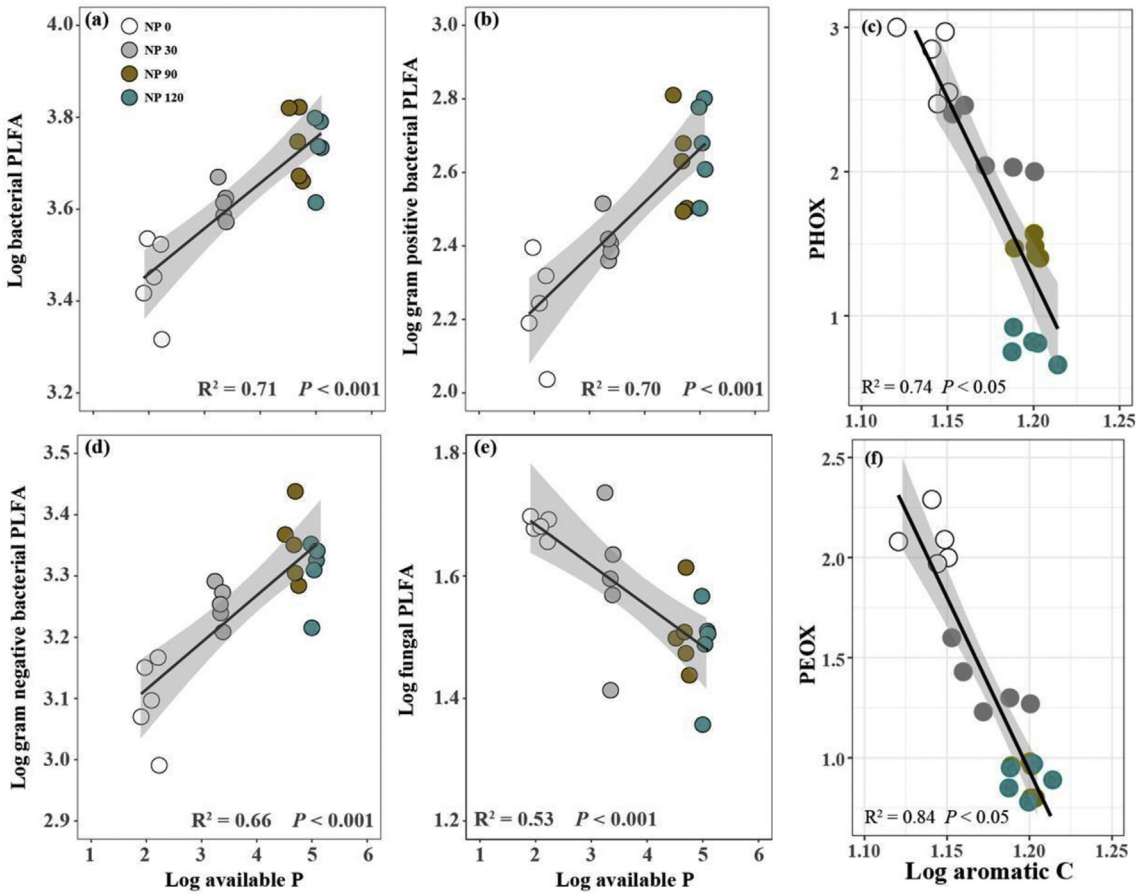

Fig. 8. Linear regressions of the bacteria (a), Gram positive bacteria (b), Gram negative bacteria (d) and fungi (e) versus available P and phenol oxidases (c) and peroxidases (f) versus aromatic C. All values are log transformed, except phenol oxidases and peroxidases. R^2 values are shown for all regressions; significances of regression are reported.

2013). Simultaneously, the breakdown of macroaggregates in our study may promote mineral crusts interaction with microbial byproducts and then formation of organo-mineral complexes (Six et al., 2004; Bischoff et al., 2017). Second, although there was no significant correlation between enzyme activities and mineral-associated C, the reduced enzyme activities could suppress the decomposition of mineral-associated C. Third, low soil pH in our study may also increase the stabilization of C in organo-metal associations through increasing mineral surface reactivity (Ye et al., 2018). Our results suggested that P availability played a critical role in determining microbial community and function, which led to increase in mineral-associated C in the alpine meadow with low P content. Similarly, Bradford et al. (2008a) also observed that P addition promoted plant-derived C transformation and increased mineral-associated C pool in low-fertility site. These results highlighted the need for P to be explicitly considered when interpreting SOC responses to increased resource availability.

4.3. Nutrient amendment increased SOC aromaticity

Generally, aromatic C was derived from lignin and recalcitrant to microbial decomposition, while alkyl C was usually derived from fatty acids, waxes, cutin, and suberin and relatively easy to decomposition (Krull et al., 2004; Suseela and Tharayil, 2018). Results from our study showed that long-term nutrient amendment increased the relative content of aromatic C but decreased the relative content of alkyl C (Fig. 6), consistent with our previous study at the same experimental site (Guo et al., 2017) and indicating nutrient addition promoted recalcitrance of SOC. A previous study reported that forest soil microbial community and function was strongly correlated with SOC chemical composition under N additions (Cusack et al., 2011). Consistent with our first hypothesis, soil microbial community shifted to bacteria dominant after nutrient amendment (Fig. 2). On the one hand, increased organic matter induced by nutrient amendment was easily used by bacterial community, thus in turn produced more microbial residue which is an important component of soil recalcitrant C (Liang et al., 2017). On the other hand, nutrient amendment could suppress the activity of oxidase, which was responsible for the decomposition of recalcitrant C, through reducing fungal biomass (Baldock et al., 1997; Berg, 2000; Frey et al., 2004).

With regard to alkyl C, there was a widely held assumption that the bacterial pathway supported high turnover rates of labile substrates such as partly labile alkyl C (Lorenz et al., 2007; Graaff et al., 2010). A previous study reported that aliphatic lipids had been degraded rapidly in soils relative to other SOM constituents in grassland ecosystem (Otto and Simpson, 2005). Therefore, relatively labile alkyl C was preferentially utilized by microorganisms, consistent with the increased bacterial biomass (Fig. S6), and thus the lignin derived aromatic C could be selectively preserved (Ekschmitt et al., 2005). Meanwhile, disintegrated aggregates increased the accessibility between bacteria and alkyl C. Our results agreed with the perception that SOC was physically protected from microbial activity regardless of its initial chemical composition (Dungait et al., 2012). However, SOC chemical composition provided a lot of ecological relevant information, especially referring to the roles of microbial community.

Understanding the mechanisms of SOC stabilization in response to nutrient enrichment are crucially important for accurately predicting and managing ecosystem C stock and associated multifunctionality. Our results supported that nutrient enrichment could exert strong bottom-

up control on SOC accumulation and stabilization, through soil physicochemical, biological or biochemical changes, and also the effects could be derived from plant community changes including species composition or functional traits that need to be measured in the future (Lavorel and Grigulis, 2011; Tamura et al., 2017). However, as our experiment was explorative we did not find direct evidence for the mechanisms of P availability on SOC stabilization process. Furthermore, our results emphasized the critically functional consequences of microbial community changes on SOC. Given the pitfalls of investigative studies in exploring cause-effect relations and the seasonal dependence of C availability for soil microbial community via plant growth phase, sophisticated design combining soil community manipulation, plant-soil feedback and temporal sampling would provide process-directed understanding the driving factors underlying SOC dynamics.

5. Conclusions

Nutrient amendment reduced soil aggregate stability but increased the mineral-associated C. The SEM results demonstrated that reduced fungal biomass and stimulated bacteria biomass derived from increased P availability promoted SOC accumulation in mineral-associated fraction with soil aggregate disintegration. Meanwhile, nutrient amendment promoted the preservation of recalcitrant aromatic C. Overall, long-term nutrient enrichment may shift the mechanisms of C stabilization from physical protection to chemical stabilization via shifting microbial community composition and function in alpine ecosystems.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2018.08.008.

References

Allison, S.D., Jastrow, J.D., 2006. Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil Biology and Biochemistry 38, 3245–3256.
Allison, S.D., Lu, Y., Weihe, C., Goulden, M.L., Martiny, A.C., Treseder, K.K., Martiny, J.B., 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94, 714–725.
Averill, C., Waring, B., 2018. Nitrogen limitation of decomposition and decay: how can it occur? Global Change Biology 24, 1417–1427.
Baldock, J.A., Oades, J.M., Nelson, P.N., Skene, T.M., Golchin, A., Clarke, P., 1997. Assessing the extent of decomposition of natural organic materials using solid-state ^{13}C NMR spectroscopy. Soil Research 35, 1139.
Berg, B., 2000. Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management 133, 13–22.
Bell, C.W., Fricks, B.E., Rocca, J.D., Steinweg, J.M., McMahon, S.K., Wallenstein, M.D., 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. Journal of Visualized Experiments Jove 81, e50961. https://doi.org/10.3791/50961.
Bischoff, N., Mikutta, R., Shibistova, O., Puzanov, A., Silanteva, M., Grebennikova, A., Fuß, R., Guggenberger, G., 2017. Limited protection of macro-aggregate-occluded organic carbon in Siberian steppe soils. Biogeosciences 14, 1–28.
Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.
Bossio, D.A., Scow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35, 265–278.
Boone, R.D., 1994. Light-fraction soil organic matter: origin and contribution to net nitrogen mineralization. Soil Biology and Biochemistry 26, 1459–1468.
Bradford, M.A., Fierer, N., Jackson, R.B., Maddox, T.R., Reynolds, J.F., 2008a. Nonlinear root-derived carbon sequestration across a gradient of nitrogen and phosphorous deposition in experimental mesocosms. Global Change Biology 14, 1113–1124.
Bradford, M.A., Fierer, N., Reynolds, J.F., 2008b. Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. Functional Ecology 22, 964–974.
Bradley, K., Drijber, R.A., Knops, J., 2006. Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 38, 1583–1595.
Bremner, J.M., Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E., 1996. Nitrogen-total. Methods of Soil Analysis. Part 3-Chemical Methods. pp. 1085–1121.
Brown, K.H., Bach, E.M., Drijber, R.A., Hofmockel, K.S., Jeske, E.S., Sawyer, J.E., Castellano, M.J., 2014. A long-term nitrogen fertilizer gradient has little effect on soil organic matter in a high-intensity maize production system. Global Change Biology 20, 1339–1350.
Camenzind, T., Hattenschwiler, S., Treseder, K.K., Lehmann, A., Rillig, M.C., 2018. Nutrient limitation of soil microbial processes in tropical forests. Ecological Monographs 88, 4–21.
Cusack, D.F., Silver, W.L., Torn, M.S., Burton, S.D., Firestone, M.K., 2011. Changes in microbial community characteristics and soil organic matter with nitrogen additions in two tropical forests. Ecology 92, 621–632.
de Vries, F.T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M.A., Bjørnlund, L., Bracht, J.H., Brady, M.V., Christensen, S., de Ruiter, P.C., 2013. Soil food web properties explain ecosystem services across European land use systems. In: Proceedings of the National Academy of Sciences of the United States of America. vol. 110. pp. 14296–14301.
Ding, J.Z., Chen, L.Y., Ji, C.J., Hugelius, G., Li, Y.N., Liu, L., Qin, S.Q., Zhang, B.B., Yang, G.B., Li, F., Fang, K., Chen, Y.L., Peng, Y.F., Zhao, X., He, H.L., Smith, P., Fang, J.Y., Yang, Y.H., 2017. Decadal soil carbon accumulation across Tibetan permafrost regions. Nature Geoscience 10, 420–424.
Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Global Change Biology 18, 1781–1796.
Ekschmitt, K., Liu, M., Vetter, S., Fox, O., Wolters, V., 2005. Strategies used by soil biota to overcome soil organic matter stability — why is dead organic matter left over in the soil? Geoderma 128, 167–176.
Frey, S.D., Knorr, M., Parrent, J.L., Simpson, R.T., 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196, 159–171.
Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid to estimate bacterial and fungal biomass in soils. Biology and Fertility of Soils 22, 59–65.
Fornara, D.A., Banin, L., Crawley, M.J., 2013. Multi-nutrient vs. nitrogen-only effects on carbon sequestration in grassland soils. Global Change Biology 19, 3848–3857.
Graaff, M.A.D., Classen, A.T., Castro, H.F., Schadt, C.W., 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytologist 188, 1055–1064.
Guo, H., Ye, C., Zhang, H., Pan, S., Ji, Y., Li, Z., Liu, M., Zhou, X., Du, G., Hu, F., 2017. Long-term nitrogen & phosphorus additions reduce soil microbial respiration but increase its temperature sensitivity in a Tibetan alpine meadow. Soil Biology and Biochemistry 113, 26–34.
Huang, X., Tang, H., Kang, W., Yu, G., Ran, W., Hong, J., Shen, Q., 2018. Redox interface-associated organo-mineral interactions: a mechanism for C sequestration under a rice-wheat cropping system. Soil Biology and Biochemistry 120, 12–23.
IPCC, 2013. Summary for policy-makers. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
Jiang, S., Liu, Y., Luo, J., Qin, M., Johnson, N.C., Opik, M., Vasar, M., Chai, Y., Zhou, X., Mao, L., Du, G., An, L., Feng, H., 2018. Dynamics of arbuscular mycorrhizal fungal community structure and functioning along a nitrogen enrichment gradient in an alpine meadow ecosystem. New Phytologist. https://doi.org/10.1111/nph.15112.
Jian, S., Li, J., Chen, J., Wang, G., Mayes, M.A., Dzantor, K.E., Hui, D., Luo, Y., 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: a meta-analysis. Soil Biology and Biochemistry 101, 32–43.
Khan, S.A., Mulvaney, R.L., Ellsworth, T.R., Boast, C.W., 2007. The myth of nitrogen fertilization for soil carbon sequestration. Journal of Environmental Quality 36, 1821–1832.
Krull, E.S., Skjemstad, J.O., Baldock, J.A., 2004. Functions of soil organic matter and the effect on soil properties. In: Project CSO 00029. GRDC, Glen Osmond, South Australia.
Lavorel, S., Grigulis, K., 2011. How fundamental plant functional trait relationships scale-up to trade-offs and synergies in ecosystem services. Journal of Ecology 100, 128–140.
LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89, 371–379.
Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 17105.
Li, J.H., Zhang, J., Li, W.J., Xu, D.H., Knops, J.M.H., Du, G.Z., 2016. Plant functional groups, grasses versus forbs, differ in their impact on soil carbon dynamics with nitrogen fertilization. European Journal of Soil Biology 75, 79–87.
Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z., Vitousek, P., Erisman, J.W., Goulding, K., Christie, P., Fangmeier, A., Zhang, F., 2013. Enhanced nitrogen

deposition over China. Nature 494, 459–463.
Liu, Y., Shi, G., Mao, L., Cheng, G., Jiang, S., Ma, X., An, L., Du, G., Collins Johnson, N., Feng, H., 2012. Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. New Phytologist 194, 523–535.
Lorenz, K., Lal, R., Preston, C.M., Nierop, K.G.J., 2007. Strengthening the soil organic carbon pool by increasing contributions from recalcitrant aliphatic bio(macro)molecules. Geoderma 142, 1–10.
Luo, Y., Qin, G., Du, G., 2006. Importance of assemblage-level thinning: a field experiment in an alpine meadow on the Tibet plateau. Journal of Vegetation Science 17, 417–424.
Mack, M.C., Schuur, E.A., Bretharte, M.S., Shaver, G.R., Chapin, F.S., 2004. Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. Nature 431, 440–443.
Mikola, J., Setälä, H., 1998. No evidence of trophic cascades in an experimental microbial-based soil food web. Ecology 79, 153–164.
Miller, R.M., Jastrow, J.D., 1990. Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biology and Biochemistry 22, 579–584.
Müller, I., Schmid, B., Weiner, J., 2000. The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. Perspectives in Plant Ecology, Evolution and Systematics 3, 115–127.
Mummey, D.L., 2006. Mycorrhizas and soil structure. New Phytologist 171, 41–53.
Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S.J., Turnbull, J., Bowman, W.D., 2002. Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419, 915–917.
Nelson, D.W., Sommers, L.E., 1996. Total carbon, organic carbon, and organic matter. Methods of Soil Analysis part 3 - Chemical methods 961–1010.
Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L. a., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939, 1–19.
Otto, A., Simpson, M.J., 2005. Degradation and preservation of vascular plant-derived biomarkers in grassland and forest soils from Western Canada. Biogeochemistry 74, 377–409.
Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G.P., Smith, P., 2016. Climate-smart soils. Nature 532, 49–57.
Plaza, C., Courtier-Murias, D., Fernández, J.M., Polo, A., Simpson, A.J., 2013. Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: a central role for microbes and microbial by-products in C sequestration. Soil Biology and Biochemistry 57, 124–134.
Riggs, C.E., Hobbie, S.E., Bach, E.M., Hofmockel, K.S., Kazanski, C.E., 2015. Nitrogen addition changes grassland soil organic matter decomposition. Biogeochemistry 125, 203–219.
Rillig, M.C., Wright, S.F., Eviner, V.T., 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant and Soil 238, 325–333.
Rosseel, Y., 2012. *lavaan*: an R package for structural equation modeling. Journal of Statistical Software 48, 1–36.
Sarker, T.C., Incerti, G., Spaccini, R., Piccolo, A., Mazzoleni, S., Bonanomi, G., 2018. Linking organic matter chemistry with soil aggregate stability: insight from ^{13}C NMR spectroscopy. Soil Biology and Biochemistry 117, 175–184.
Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.
Silveira, M.L., Liu, K., Sollenberger, L.E., Follett, R.F., Vendramini, J.M.B., 2013. Short-term effects of grazing intensity and nitrogen fertilization on soil organic carbon pools under perennial grass pastures in the southeastern USA. Soil Biology and Biochemistry 58, 42–49.
Suseela, V., Tharayil, N., 2018. Decoupling the direct and indirect effects of climate on plant litter decomposition: accounting for stress-induced modifications in plant chemistry. Global Change Biology 24, 1428–1451.
Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research 79, 7–31.
Six, J., Elliott, E.T., Paustian, K., Doran, J.W., 2010. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal 62, 1367–1377.
Spaccini, R., Mbagwu, J.S.C., Conte, P., Piccolo, A., 2006. Changes of humic substances characteristics from forested to cultivated soils in Ethiopia. Geoderma 132, 9–19.
Stewart, C.E., Plante, A.F., Paustian, K., Conant, R.T., Six, J., 2008. Soil carbon saturation: linking concept and measurable carbon pools. Soil Science Society of America Journal 72, 379–392.
Suding, K.N., Collins, S.L., Gough, L., Clark, C., Cleland, E.E., Gross, K.L., Milchunas, D.G., Pennings, S., 2005. Functional- and abundance-based mechanisms explain diversity loss due to N fertilization. Proceedings of the National Academy of Sciences of the United States of America 102, 4387–4392.
Tamura, M., Suseela, V., Simpson, M., Powell, B., Tharayil, N., 2017. Plant litter chemistry alters the content and composition of organic carbon associated with soil mineral and aggregate fractions in invaded ecosystems. Global Change Biology 23, 4002–4018.
Treseder, K.K., Allen, E.B., Egerton-Warburton, L.M., Hart, M.M., Klironomos, J.N., Maherali, H., Tedersoo, L., 2018. Arbuscular mycorrhizal fungi as mediators of ecosystem responses to nitrogen deposition: a trait-based predictive framework. Journal of Ecology 106, 480–489.
Wang, Q., Wang, S., He, T., Liu, L., Wu, J., 2014. Response of organic carbon mineralization and microbial community to leaf litter and nutrient additions in subtropical forest soils. Soil Biology and Biochemistry 71, 13–20.
Wershaw, Robert, L., 1999. Molecular aggregation of humic substances. Soil Science 164, 803–813.
Xu, J., Liu, S., Song, S., Guo, H., Tang, J., Yong, J.W.H., Ma, Y., Chen, X., 2018. Arbuscular mycorrhizal fungi influence decomposition and the associated soil microbial community under different soil phosphorus availability. Soil Biology and Biochemistry 120, 181–190.
Yang, Z., Ruijven, J.V., Du, G., 2011. The effects of long-term fertilization on the temporal stability of alpine meadow communities. Plant and Soil 345, 315–324.
Ye, C.L., Chen, D.M., Hall, S.J., Pan, S., Yan, X.B., Bai, T.S., Guo, H., Zhang, Y., Bai, Y.F., Hu, S.J., Reynolds, H., 2018. Reconciling multiple impacts of nitrogen enrichment on soil carbon: plant, microbial and geochemical controls. Ecology Letters. https://doi.org/10.1111/ele.13083.
Yu, G., Xiao, J., Hu, S., Polizzotto, M.L., Zhao, F.J., Mcgrath, S.P., Li, H., Ran, W., Shen, Q., 2017. Mineral availability as a key regulator of soil carbon storage. Environmental Science and Technology 51, 4960–4969.
Zak, D.R., Freedman, Z.B., Upchurch, R., Steffens, M., Kögelknabner, I., 2017. Anthropogenic N deposition increases soil organic matter accumulation without altering its biochemical composition. Global Change Biology 23, 933–944.